

# The physiological basis for regeneration response to variable retention harvest treatments in three pine species

MATTHEW D. POWERS<sup>1,4\*</sup>, KURT S. PREGITZER<sup>2,5</sup>, BRIAN J. PALIK<sup>3</sup> AND CHRISTOPHER R. WEBSTER<sup>1</sup>

<sup>1</sup> School of Forest Resources and Environmental Science, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, USA

<sup>2</sup> Department of Natural Resources and Environmental Science, University of Nevada, 1000 Valley Road, Reno, NV 89512, USA

<sup>3</sup> USDA Forest Service Northern Research Station, 1831 Hwy 169 East, Grand Rapids, MN 55744, USA

<sup>4</sup> Present address: USDA Forest Service Northern Research Station, 1831 Hwy 169 East, Grand Rapids, MN 55744, USA

<sup>5</sup> Present address: College of Natural Resources, University of Idaho, PO Box 441142, Moscow, ID 83844, USA

\*Corresponding author. E-mail: mdpowers@mtu.edu

## Summary

Variable retention harvesting (VRH) is promoted for enhancing biodiversity and ecosystem processes in managed forests, but regeneration responses to the complex stand structures that result from VRH are poorly understood. We analyzed foliar stable carbon isotope ratios ( $\delta^{13}\text{C}$ ), oxygen isotope ratios ( $\delta^{18}\text{O}$ ), light-saturated photosynthetic rates ( $A_{\text{max}}$ ), stomatal conductance ( $g_s$ ) and sizes of pine seedlings in *Pinus resinosa* stands treated with VRH systems that created different spatial patterns of overstorey retention. *Pinus banksiana*, *P. resinosa* and *Pinus strobus* seedlings in dispersed retention, aggregated retention between small gaps and aggregated retention between large gaps treatments had greater  $\delta^{13}\text{C}$ ,  $A_{\text{max}}$ ,  $g_s$ , diameter and height than seedlings in unharvested treatments, but seedling performance was similar among VRH treatments when averaged across stands. There were no significant differences in  $\delta^{18}\text{O}$  associated with any treatment. These results indicate seedling photosynthetic capacity, water relations and growth were improved by VRH, but the spatial pattern of retention had surprisingly little impact on average performance in the first 4 years after harvesting. This suggests retention treatments that produce different residual stand structures can promote similar mean rates of early seedling development when performance is averaged across all of the neighbourhood environments within each treatment.

## Introduction

Forest management strategies increasingly call for enhancing or conserving the structural complexity and biodiversity of managed stands to better emulate the outcomes of natural disturbance and stand development (Coates and Burton, 1997; Franklin *et al.*, 2002; Palik *et al.*, 2002). Variable retention harvest systems have been proposed as an approach to emulate natural disturbances (Franklin *et al.*, 2000), but there is limited evidence to use in predicting developmental responses to the complex stand structures produced by different spatial patterns of retention. Much of the discussion surrounding the effects of variable reten-

tion harvesting (VRH) on tree regeneration, for instance, has focused on how different spatial patterns of overstorey retention influence resource availability, tree establishment, survival and growth (Palik *et al.*, 1997; Halpern *et al.*, 1999; Palik *et al.*, 2003; Maguire *et al.*, 2006; Sullivan *et al.*, 2008), but information about seedling physiological performance following VRH would also provide valuable insight into the mechanisms driving regeneration dynamics in complex, spatially variable stand structures.

Light and soil moisture availability are often greater following partial cuttings or in canopy gaps than in undisturbed forests (Palik *et al.*, 1997; Burgess and Wetzels, 2000; Wetzels and Burgess, 2001; Prescott *et al.*, 2003;

Skov *et al.*, 2004; Ritter *et al.*, 2005; Oguchi *et al.*, 2006; Boucher *et al.*, 2007). Leaves in high light environments typically have greater light-saturated photosynthetic rates ( $A_{\max}$ ) than those in lower light environments (Ellsworth and Reich, 1993; Bond *et al.*, 1999), and seedlings respond to increases in light availability by increasing  $A_{\max}$  (Pothier and Prevost, 2002; Oguchi *et al.*, 2006). Foliar nitrogen concentrations can also increase as overstorey density declines following partial overstorey harvesting (Wetzel and Burgess, 2001; Boucher *et al.*, 2007; Dumais and Prevost, 2008), and foliar nitrogen has a positive relationship with  $A_{\max}$  (Evans, 1983; Reich *et al.*, 1995). Low water availability, on the other hand, can reduce stomatal conductance ( $g_s$ ) (Comstock and Ehleringer, 1984; Dang *et al.*, 1997), which limits photosynthesis (Comstock and Ehleringer, 1984; Maier and Teskey, 1992; Dang *et al.*, 1997). Thus, we might expect differences in average seedling performance among treatments that create different overstorey structures and therefore different distributions of resource availability across stands (Palik *et al.*, 1997, 2003).

Dense understorey competition may limit increases in resource availability resulting from harvesting, which could alter the expected responses of tree regeneration to VRH. Reducing understorey competition after partial harvesting can increase light, nitrogen and soil moisture availability (Palik *et al.*, 1997; McGuire *et al.*, 2001; Wetzel and Burgess, 2001; Palik *et al.*, 2003; Boucher *et al.*, 2007). This suggests VRH treatments that incorporate some form of understorey competition control should promote faster seedling growth than overstorey harvesting alone.

Stable isotopes of carbon and oxygen in plant tissues provide information about carbon–water relations that should be useful in understanding the effects of complex stand structures on seedling physiological performance. The stable carbon isotope ratio ( $\delta^{13}\text{C}$ ) of C3 plants is inversely proportional to the ratio of the intercellular  $\text{CO}_2$  concentration in a leaf ( $c_i$ ) to the concentration of  $\text{CO}_2$  in the atmosphere ( $c_a$ ), which leads to a positive correlation between  $\delta^{13}\text{C}$  and the ratio of  $A_{\max}/g_s$  (Farquhar *et al.*, 1982, 1989). The stable oxygen isotope ratio ( $\delta^{18}\text{O}$ ) of plant tissues is thought to reflect evaporative processes within the leaf. Under well-mixed conditions, leaf water enrichment is proportional to  $1 - e_a/e_i$ , where  $e_a$  is the water vapour pressure in the atmosphere and  $e_i$  is the water vapour pressure in the leaf (Dongmann *et al.*, 1974; Farquhar and Lloyd, 1993; Barbour, 2007). Transpiration ( $E$ ) decreases as  $e_a/e_i$  increases, which leads to a positive correlation between  $\delta^{18}\text{O}$  and  $E$  when differences in  $E$  are primarily driven by changes in evaporative demand. Reductions in  $g_s$ , however, increase leaf water enrichment due to kinetic fractionation and decrease  $E$ , which may increase  $e_i$  due to reduced evaporative cooling of the leaf (Farquhar *et al.*, 2007). This suggests tissue  $\delta^{18}\text{O}$ , which incorporates oxygen from leaf water, should increase as  $g_s$  decreases in plants growing in environments where changes in  $g_s$  are primarily responsible for differences in  $E$  (DeNiro and Epstein, 1979; Yakir, 1992; Farquhar *et al.*, 2007).

We evaluated the physiological responses of three species of pine seedlings to VRH treatments to investigate the

mechanisms that drive differences in mean, stand-scale performance among treatments that retain different spatial patterns of residual overstorey trees. We compared average seedling responses with VRH treatments applied to whole stands using stable isotope techniques as well as responses to specific neighbourhood environments created within each VRH treatment using gas exchange measurements. We hypothesized that (1) seedling foliage from VRH treatments should have greater  $\delta^{13}\text{C}$  and lower  $\delta^{18}\text{O}$  than foliage from unharvested reference stands because retention harvesting should increase light and soil moisture availability, leading to increases in seedling  $A_{\max}$  and  $g_s$ ; (2) seedling  $A_{\max}$  and  $g_s$  should decrease as the neighbourhood environment shifts along a gradient from large canopy gaps to smaller gaps to a partial canopy to a closed canopy due to declines in light and soil moisture availability and (3) seedling foliage from understorey release treatments should have greater  $\delta^{13}\text{C}$ ,  $A_{\max}$  and  $g_s$  but lower  $\delta^{18}\text{O}$  than foliage from untreated understories because understorey release treatments should increase light and soil moisture availability. While a primary objective of VRH treatments is to increase within-stand variability, we focus here on mean stand-scale performance to help inform natural resource managers about productivity tradeoffs between different stand structures and VRH systems.

## Materials and methods

### Study sites

This study is part of the Red Pine Retention Study, a long-term silvicultural experiment testing the effects of various spatial patterns of overstorey retention and understorey competition control in managed *Pinus resinosa* Ait. forests (Palik and Zasada, 2003; Palik *et al.*, 2005). The study sites are located on the Chippewa National Forest in north-central Minnesota, USA. The area is characterized by a continental climate, with warm summers and cool winters. Soils are well-drained, coarse-textured sands consisting of mixed, frigid Aquic Udipsamments, mixed, frigid Lamellic Udipsamments and mixed, frigid Typic Udipsamments with little topographic variability (Richardson, 1997).

The study has a randomized complete block design with four replicates. Each block includes four stands, each randomly assigned one of four harvesting treatments including dispersed retention, aggregated retention between small (0.1 ha) gaps, aggregated retention between large (0.3 ha) gaps and reference (no harvesting). Harvesting in the dispersed retention treatments left residual trees spaced more or less evenly throughout the stands. In the aggregated retention treatments, an average of either 28 small (0.1 ha) gaps or 18 large (0.3 ha) gaps were cut per stand and the matrix between gaps was thinned lightly. Each VRH treatment reduced residual basal areas to  $\sim 16 \text{ m}^2 \text{ ha}^{-1}$ , while reference stands averaged  $36 \text{ m}^2 \text{ ha}^{-1}$ . Stand size averaged 16 ha. The overstorey consisted of a single stratum, with top heights for canopy trees averaging 23 m during this study. *P. resinosa* accounted for 90 per cent of the basal

area of stems greater than 2.5 cm d.b.h., while *Pinus strobus* L., *Betula papyrifera* Marsh., *Quercus rubra* L. and *Abies balsamea* (L.) Mill. each represented 1–2 per cent of the basal area. Dense patches of the shrub *Corylus cornuta* Marsh. were common in the understorey.

Half of each stand was assigned an understorey release treatment in which woody shrubs and aspen regeneration (*Populus* spp.) over 0.3 m in height were cut with brush saws. VRH treatments were installed in the winter of 2002–2003, and understorey release treatments were applied every spring beginning in 2003. Equal numbers of seedlings of *Pinus banksiana* Lamb., *P. resinosa* and *P. strobus* were planted as 2–0 bare-root stock at a spacing of  $2.7 \times 2.7$  m, (1300 seedlings ha<sup>-1</sup>) throughout the retention harvesting treatments and around permanent sampling points in the reference overstorey treatments in the spring of 2003. Planted seedlings were used because natural seed production of *P. resinosa* is sporadic, and few *P. banksiana* or *P. strobus* seed trees were present.

Seedling diameter averaged 0.76 cm (0.031 SE) for *P. banksiana*, 0.59 cm (0.032 SE) for *P. resinosa* and 0.66 cm (0.029 SE) for *P. strobus* at the time of planting, and seedling height averaged 12.99 cm (0.31 SE) for *P. banksiana*, 16.81 cm (0.23 SE) for *P. resinosa* and 17.44 cm (0.21 SE) for *P. strobus* with no differences in initial seedling size among treatments. Since there were no differences in the size of planted seedlings among treatments, we assume that any size differences at the time of our measurements are indicative of differences in growth increment following planting. Seedling size measurements described below were taken during the fifth growing season after harvesting (2007), unless otherwise indicated.

#### Stable isotope and size sampling

Foliar samples were collected from one *P. banksiana*, *P. resinosa* and *P. strobus* at five sampling points in both the understorey reference and understorey release half of each stand. Research suggests only four to five trees of a given species are needed to capture within-site isotopic variability and produce representative and repeatable means for the site (Leavitt and Long, 1984, 1986). Sampling points in the overstorey reference and dispersed retention treatments were selected randomly, but points in the aggregated retention treatments were selected to represent the range of overstorey conditions in these treatments including one gap interior, two gap-aggregate edge and two aggregate interior points in each understorey treatment per stand. Foliage formed the previous growing season (2006) was collected from throughout the crown of each tree for analyses of  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and mass-based foliar nitrogen concentration ( $N_{\text{mass}}$ ). Stem diameter above the root collar and seedling height were measured on each tree before bud break in the spring of 2007.

Foliar samples were dried and pulverized on a ball mill for isotopic and elemental analysis. Foliar nitrogen and  $\delta^{13}\text{C}$  were analyzed at the Michigan Technological University Forest Ecology Stable Isotope Laboratory. Oxygen isotope ratios were measured at the Washington State University Stable Isotope. Cellulose is often isolated for isotopic

studies, but whole tissue and cellulose provide similar information about environmental controls on stable isotope signatures in the species considered here (Powers *et al.*, 2008a) so our isotopic data are from whole foliage samples.

#### Physiological measurements

Gas exchange data were collected from the *P. banksiana*, *P. resinosa* and *P. strobus* seedling nearest to one sampling point in the understorey reference half of each stand and a second sampling point in the understorey release half. Study points used for gas exchange measurements were located near the centre of gaps in the aggregated retention treatments, and in the matrix of the dispersed retention (partial canopy) and overstorey control (closed canopy) treatments in order to represent the contrast between open (gap) and partial canopy environments thought to promote differences in stand-scale performance among VRH treatments (Palik *et al.*, 1997, 2003). Rates of  $A_{\text{max}}$  and  $g_s$  were measured on 1-year old (2006) needles using a portable gas exchange system (LI-6400; Li-Cor Biosciences, Lincoln, NE) with a 6 cm<sup>2</sup> cuvette and a red/blue light source set to provide a constant irradiance of 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

Gas exchange measurements were made between 0830 and 1100 h on cloudless or mostly sunny days over 1 week in early June, July and August. All seedlings in a treatment block were measured on the same day of sampling. Two fascicles (four needles) of *P. banksiana* and *P. resinosa* or one fascicle (five needles) of *P. strobus* were removed from each seedling and immediately enclosed in the LI-6400's cuvette. Gas exchange rates from detached foliage are generally similar to those of intact foliage for several minutes after removal in pines (Dang *et al.*, 1997; Nagel and O'Hara, 2001; Boucher *et al.*, 2007). Two readings of leaf diameter were taken from the midpoint of each needle and used to compute the all-sided leaf area inside the cuvette based on the assumption that the surface area of each needle can be estimated as sections of a cylinder (Johnson, 1984; Svenson and Davies, 1992). Gas exchange data were recalculated using these estimates of all-sided leaf area.

#### Statistical methods

Seedling size and  $N_{\text{mass}}$  data were analyzed using a split-split plot analysis of variance (ANOVA) with overstorey treatment as the whole plot, understorey treatment as the split plot, species as the split-split plot and treatment area (replicate) as a blocking variable. Species-related differences in mesophyll conductance can influence  $\delta^{13}\text{C}$  (Seibt *et al.*, 2008) and differences in source water isotopic composition associated with different patterns of root development could influence  $\delta^{18}\text{O}$ , so isotopic data was analyzed using separate split-plot ANOVAs for each species. Overstorey treatment means were compared with the following set of orthogonal contrasts: overstorey reference *vs* VRH treatments, dispersed retention *vs* aggregated retention treatments and aggregated with large gaps *vs* aggregated with small gaps. All other comparisons of means were made using Tukey's test.



The stratified sampling we utilized in the aggregated retention treatments could produce results that over-represent the influence of a particular neighbourhood environment relative to the true distribution of these environments. We analyzed the  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ,  $N_{\text{mass}}$ , height and diameter data using weighting for the two aggregated retention treatments to evaluate potential bias. Weights were based on the proportion of study points falling into five strata that spanned the range of basal area conditions relative to the actual proportion of each treatment represented by each of the five strata. Weighted means were very similar to the unweighted means (Table 1), and results from the statistical analyses using weighted and unweighted data were identical so we only present results from analyses using the original unweighted data.

Preliminary analyses suggested trends in gas exchange variables were similar among measurement periods, so gas exchange data were averaged across the three measurement periods and analyzed using split-split plot ANOVA with overstorey treatment as the whole plot factor, understorey treatment as the split plot factor, species as the split-split plot factor and replicate as a blocking variable. Predawn xylem pressure potential was analyzed using split-plot ANOVA. The following orthogonal contrasts were used to examine differences between neighbourhood environments: closed canopy *vs* partial canopy and gap environments, partial canopy *vs* pooled gap environments and large gap *vs* small gap environments. Significant interactions and differences between species were evaluated using Tukey's HSD. Regression and ANOVA assumptions regarding normality were checked using normal probability plots of error residuals and we tested for heteroscedasticity using both plots of error residuals against predicted values and White's test (White, 1980). All statistical tests were performed using SAS (SAS Institute, Cary, NC; Version 9.0) at a significance level of  $\alpha = 0.05$ .

## Results

### Stable isotopes and foliar nitrogen

Foliar  $\delta^{13}\text{C}$  was greater (less negative) in VRH treatments than overstorey reference stands for *P. banksiana*, *P. resinosa*

and *P. strobus* ( $P = 0.003$ ,  $P = 0.001$  and  $P < 0.001$ , respectively), but there were no significant differences among VRH treatments (Figure 1). VRH treatments were enriched 1.7–3.0‰ compared with the overstorey reference, but the differences among VRH treatments ranged from only 0.2–0.8‰. Understorey release treatments were also associated with greater  $\delta^{13}\text{C}$  than understorey reference treatments for *P. banksiana*, *P. resinosa* and *P. strobus* ( $P = 0.027$ ,  $P = 0.017$  and  $P = 0.004$ , respectively), but the effect of understorey treatment (0.5–0.7‰) was negligible compared with the effect of harvesting. The overstorey treatment by understorey treatment interaction was not significant for any species.

There were no significant differences in foliar  $\delta^{18}\text{O}$  among overstorey treatments, understorey treatments or their interaction for any species (Figure 1). Although no factors were significant at the  $\alpha = 0.05$  level in the ANOVA analyses, it may be worth noting that contrast comparing overstorey reference to VRH treatments approached significance for *P. banksiana* ( $P = 0.073$ ) and was significant for *P. resinosa* ( $P = 0.009$ ). The differences between overstorey reference and VRH treatments ranged from 0.6 to 1.0‰ for *P. banksiana* and 1.5–1.6‰ for *P. resinosa* but only 0.0–0.2‰ for *P. strobus*. Differences in  $\delta^{18}\text{O}$  among VRH treatments ranged from only 0.0–0.4‰, regardless of species.

Foliar nitrogen concentrations varied significantly among species ( $P < 0.001$ ), but not by overstorey treatment, understorey treatment or any interaction involving these terms (Figure 2).  $N_{\text{mass}}$  was greatest in *P. banksiana*, intermediate in *P. strobus* and lowest in *P. resinosa*.

### Seedling size

Seedling height was influenced by overstorey treatment ( $P < 0.001$ ), understorey treatment ( $P = 0.046$ ) and species ( $P < 0.001$ ), but there were no significant interactions among these factors (Figure 2). Seedlings in VRH treatments were 80–103 per cent taller than seedlings in unharvested controls but there were no significant differences among VRH treatments where average heights varied by only 6–13 per cent. Seedlings in understorey release treatments were significantly taller than those in understorey

Table 1: Differences between weighted and unweighted means for stable carbon isotope ratios ( $\delta^{13}\text{C}$ ), stable oxygen isotope ratios ( $\delta^{18}\text{O}$ ), mass-based foliar nitrogen concentrations ( $N_{\text{mass}}$ ), diameter (DIA) and height (HT) of pine seedlings growing in stands harvested using aggregated retention between small (0.1 ha) gaps or aggregated retention between large (0.3 ha) gaps

	<i>Pinus banksiana</i>		<i>Pinus resinosa</i>		<i>Pinus strobus</i>		All species combined		
Treatment	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$N_{\text{mass}}$	DIA	HT
Aggregated – small gaps	0.518	0.550	0.358	4.632	0.280	1.961	1.776	1.043	3.973
Aggregated – large gaps	0.009	0.41	0.099	0.683	0.147	0.999	1.056	1.190	4.967
Understorey reference	0.148	0.213	0.194	1.857	0.081	0.205	2.321	0.913	0.212
Understorey release	0.106	1.768	0.028	0.160	0.016	1.544	2.004	1.124	0.839

Overstorey treatments were paired with either the removal of woody competition (understorey release) or no understorey manipulation (understorey reference). Values represent the difference between weighted and unweighted means expressed as a percentage of the unweighted means.

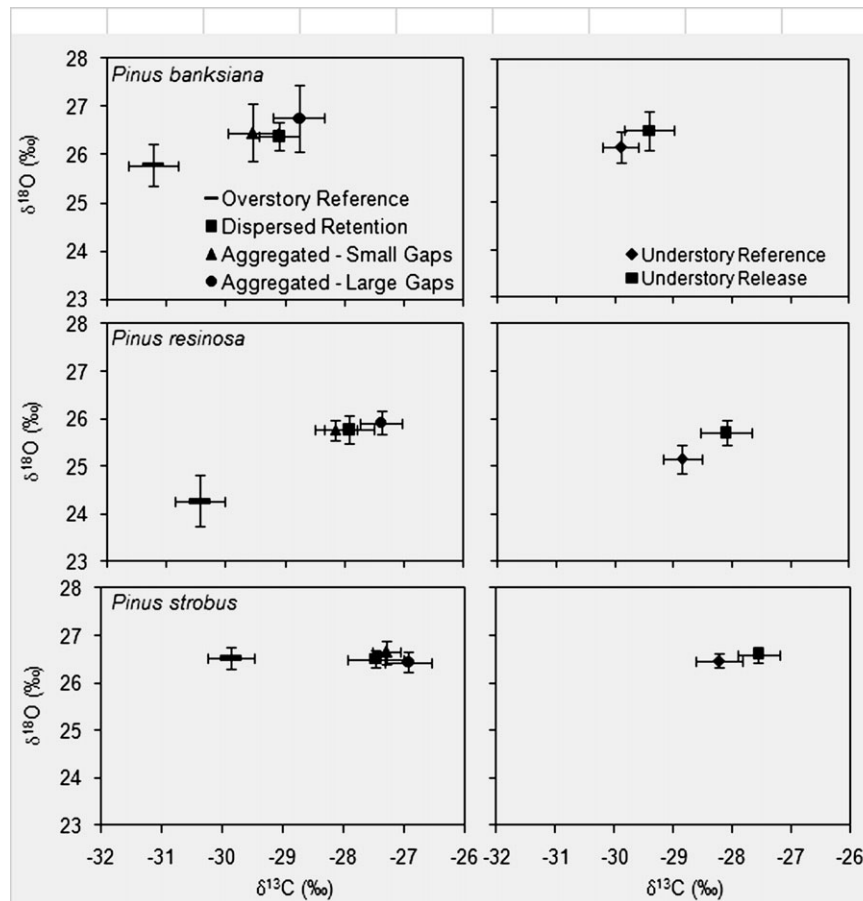


Figure 1. Relationships between stable carbon ( $\delta^{13}\text{C}$ ) and oxygen ( $\delta^{18}\text{O}$ ) isotope ratios in jack pine (*Pinus banksiana*), red pine (*Pinus resinosa*) and eastern white pine (*Pinus strobus*) seedlings from stands harvested using dispersed retention, aggregated retention between 0.1 ha gaps (small gaps), aggregated retention between 0.3 ha gaps (large gaps) or left unharvested (overstorey reference). Overstorey treatments were paired with either the removal of woody competition (understorey release) or no understorey manipulation (understorey reference). Data points represent treatment averages. Bars represent 1 SE.

reference treatments, but the difference was small (~15 per cent). Among species, heights were ranked *P. banksiana* > *P. strobus* > *P. resinosa*.

Seedling diameter varied with overstorey treatment ( $P < 0.001$ ), understorey treatment ( $P < 0.001$ ) and species ( $P < 0.001$ ), but the effect of understorey treatment was not consistent across overstorey treatments (overstorey  $\times$  understorey;  $P = 0.025$ ) or species (understorey  $\times$  species;  $P = 0.048$ , Table 2) and the effect of species varied with overstorey treatment (overstorey  $\times$  species;  $P = 0.001$ ). Seedlings in VRH treatments were larger in diameter than seedlings in the overstorey reference ( $P < 0.001$ ) regardless of understorey treatment or species, but there were no significant differences in diameter among VRH treatments (Figure 2). *P. banksiana* seedlings were largest in diameter in both understorey treatments. *P. strobus* were larger than *P. resinosa* in the understorey release treatment, but there was no significant difference between these species in the understorey reference. In VRH treatments, *P. bank-*

*siana* seedlings were larger in diameter than *P. strobus*, which were larger than *P. resinosa*, but there were no differences in diameter among species in overstorey reference stands. Seedling diameters in VRH treatments were 76–97 per cent larger than diameters in the overstorey reference, but seedling diameter varied by only 4–12 per cent among VRH treatments and by ~25 per cent between understorey treatments.

#### Seedling gas exchange

Mean growing season  $A_{\text{max}}$  varied with neighbourhood environment ( $P = 0.039$ ) and species ( $P < 0.001$ ), but the effect of neighbourhood environment varied by understorey treatment ( $P = 0.009$  for the interaction). There were no significant differences in  $A_{\text{max}}$  associated with understorey treatment or any interactions involving species. Mean growing season  $A_{\text{max}}$  was significantly higher in gaps and

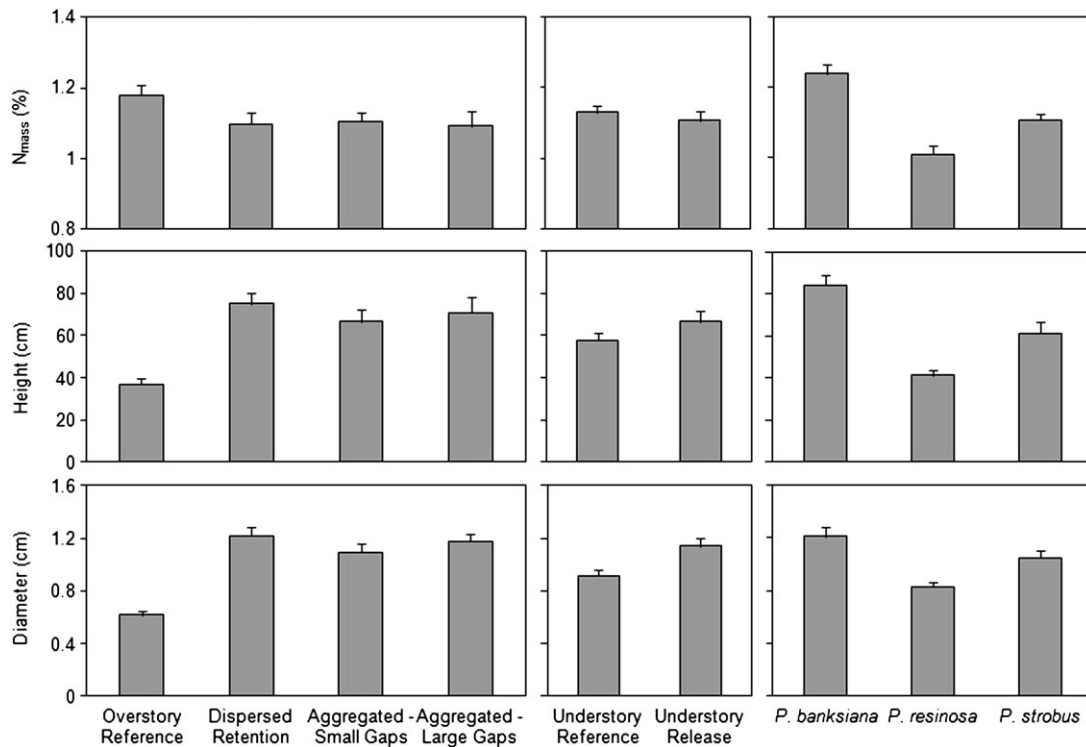


Figure 2. Foliar nitrogen concentration ( $N_{mass}$ ), diameter and height of seedlings from jack pine (*Pinus banksiana*), red pine (*Pinus resinosa*) and eastern white pine (*Pinus strobus*) seedlings planted in stands harvested using dispersed retention, aggregated retention between 0.1 ha gaps (small gaps), aggregated retention between 0.3 ha gaps (large gaps) or left unharvested (overstorey reference). Overstorey treatments were paired with either the removal of woody competition (understorey release) or no understorey manipulation (understorey reference). Significant interactions between overstorey treatment and understorey treatment or species were also present for seedling diameter (Table 2). Bars represent 1 SE.

Table 2: Differences in diameter (centimetre) of jack pine (*Pinus banksiana*), red pine (*Pinus resinosa*) and eastern white pine (*Pinus strobus*) seedlings between understorey competition control treatments

Treatment		Understorey reference	Understorey release
Overstorey treatment	Overstorey reference	0.580 (0.017)	0.661 (0.047)
	Dispersed retention*	1.052 (0.071)	1.389 (0.075)
	Aggregated – small gaps*	0.959 (0.062)	1.238 (0.094)
	Aggregated – large gaps*	1.076 (0.074)	1.276 (0.082)
Species	<i>P. banksiana</i> *	1.089 (0.088)	1.330 (0.105)
	<i>P. resinosa</i>	0.765 (0.039)	0.890 (0.057)
	<i>P. strobus</i> *	0.890 (0.057)	1.203 (0.082)

Values in parentheses indicate one standard error

\* Indicates the presence of significant understorey treatment effects.

partial canopy environments than in closed canopy environments regardless of understorey treatment (Figure 3), but differences between partial canopy and gap environments were dependent upon understorey treatment. In the understorey release treatment,  $A_{max}$  was 27–39 per cent higher in small gaps than in the large gap and partial canopy environments, but there were no significant differences in  $A_{max}$  among gap and partial canopy environments within understorey control treatments where the differences among these environments ranged from only 4–16 per cent (Table 3). Averaged across species and understorey treatments,  $A_{max}$  was 81–101 per cent higher in par-

tial canopy and gap environments compared with closed canopy environments but varied by only 11 per cent among partial canopy and gap environments. Photosynthetic rates were highest for *P. banksiana* seedlings, but there were no significant differences in  $A_{max}$  between *P. resinosa* and *P. strobus* seedlings (Figure 3).

Stomatal conductance was also influenced by neighbourhood environment ( $P = 0.011$ ) but not by understorey treatment, species or any interaction between these three variables. Conductance was 30–69 per cent higher in partial canopy and gap environments compared with closed canopy environments and 25 per cent higher in small gaps

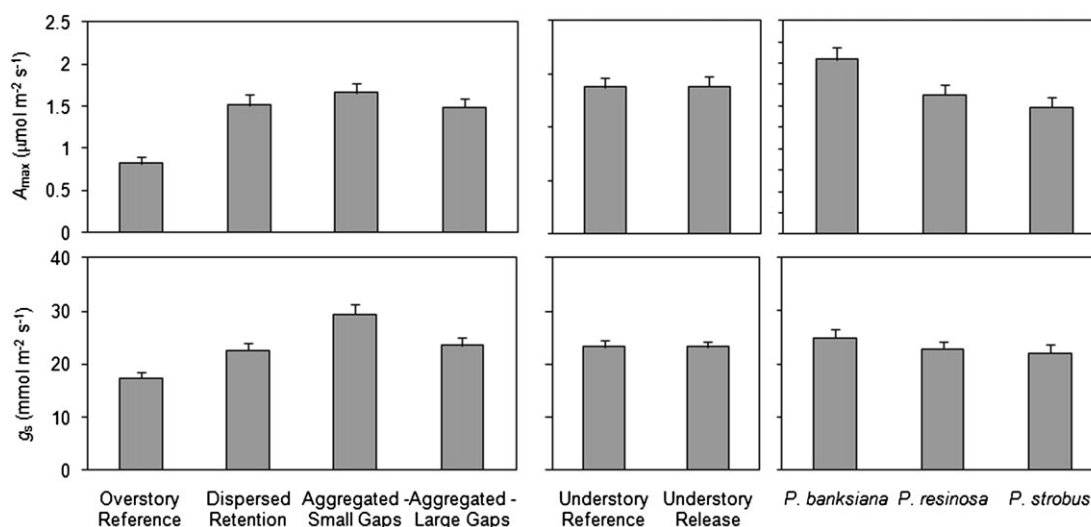


Figure 3. Light-saturated photosynthetic rates ( $A_{\max}$ ) and stomatal conductance ( $g_s$ ) of jack pine (*Pinus banksiana*), red pine (*Pinus resinosa*) and eastern white pine (*Pinus strobus*) seedlings in closed canopy, partial canopy, small gap (0.1 ha) or large gap (0.3 ha) environments in red pine stands. Plots were treated with either the removal of woody competition (understorey release) or no understory manipulation (understorey reference). Significant interactions between overstorey treatment and understory treatment were also present for  $A_{\max}$  (Table 3). Bars represent 1 SE.

Table 3: Light-saturated photosynthetic rates ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) of *Pinus* seedlings associated with different structural environments and understory competition control treatments

Structural environment	Understorey reference	Understorey release
Closed canopy	0.863 (0.121)	0.782 (0.097)
Partial canopy	1.681 (0.203)	1.344 (0.138)
Small gap (0.1 ha)	1.445 (0.125)	1.876 (0.173)
Large gap (0.3 ha)	1.502 (0.144)	1.476 (0.143)

Values in parentheses indicated 1 SE.

than large gaps (Figure 3). There were no significant differences in  $g_s$  between large gap and partial canopy environments.

## Discussion

In general, VRH appeared to increase seedling gas exchange and growth compared with uncut reference stands, but average seedling responses were surprisingly similar among the three VRH treatments themselves. As predicted, foliar  $\delta^{13}\text{C}$  was higher in VRH treatments than in the overstorey reference, but no species showed strong evidence of lower foliar  $\delta^{18}\text{O}$  in VRH treatments (Hypothesis 1), and there were no significant isotopic differences among VRH treatments in any species. Gas exchange parameters did not show the expected decrease from large gaps to small gaps to partial canopy environments (Hypothesis 2) and generally showed little variability except between closed canopy and partial canopy or gap environments. Understorey

release treatments were associated with the predicted increase in  $\delta^{13}\text{C}$ , but there was no direct evidence of an increase in  $A_{\max}$ ,  $g_s$  or  $\delta^{18}\text{O}$  (Hypothesis 3), and the understorey treatment effect on  $\delta^{13}\text{C}$  and seedling size was small compared with overstorey treatment effects.

We found little evidence of differences in mean, stand-scale physiological performance or growth among the three VRH treatments. Although different spatial patterns of tree retention are expected to produce variable stand-scale responses (Palik *et al.*, 1997; Halpern *et al.*, 1999; Palik *et al.*, 2003; Mitchell *et al.*, 2007), the absence of large physiological differences between gap and partial canopy environments may explain why differences in mean, stand-scale seedling performance were so small among the three VRH treatments in our study. Greater stand-scale growth may be expected in aggregated retention treatments because seedling performance typically declines in a negative exponential pattern as overstorey abundance increases so treatments that increase the proportion of neighbourhood environments characterized by very low overstorey competition (e.g. large gaps) should maximize average growth at the stand scale (Palik *et al.*, 1997; Acker *et al.*, 1998; Palik *et al.*, 2003; Mitchell *et al.*, 2007). The finding that seedling gas exchange was similar in large gaps and partial canopy environments with moderate basal areas suggests these neighbourhood environments were not separated by a large enough distance on the competition curve to foster differences in average stand-scale seedling growth.

While this explanation conflicts somewhat with previous studies in this system that have demonstrated the potential for varying seedling performance in different neighbourhood environments (i.e. Powers *et al.*, 2008b,

2009), it is consistent with the very small differences in mean isotopic and size indices we found among VRH treatments at the stand scale. Additionally, planted seedling survival varied little across the three VRH treatments (B. Palik, unpublished data), suggesting that there were no significant differences in stress among the three residual stand structures. Seedling gas exchange data did provide limited evidence of differences among specific environments created by different VRH treatments but only within understorey release treatments. This may suggest that any potential for differential seedling performance across VRH treatments (which encompassed both understorey reference and release treatments in each stand) was overshadowed by resource limitations associated with understorey competition (Palik *et al.*, 1997; McGuire *et al.*, 2001; Wetzel and Burgess, 2001; Palik *et al.*, 2003; Boucher *et al.*, 2007), although the absence of interactions between overstorey and understorey treatments in stand-scale analyses of VRH treatment effects and relatively small understorey treatment effects indicate this is an unlikely explanation for the lack of large differences among VRH treatments.

The high foliar  $\delta^{13}\text{C}$  we found in VRH treatments relative to reference stands suggests retention harvesting decreased  $c_i$ , which would result from an increase in the rate of  $\text{CO}_2$  consumption relative to the rate of  $\text{CO}_2$  diffusion into the leaf caused by an increase in the ratio of photosynthesis to  $g_s$  (Farquhar *et al.*, 1982, 1989). The absence of large differences in foliar  $\delta^{18}\text{O}$  suggests the differences in  $E$  among overstorey treatments were minimal at the stand scale. This could indicate the difference in foliar  $\delta^{13}\text{C}$  we observed between overstorey controls and retention treatments was driven largely by greater photosynthetic rates within retention treatments than in overstorey controls rather than by differences in  $g_s$ .

Since harvesting can increase both soil moisture (Dunlap and Helms, 1983; Aussenac and Granier, 1988; Breda *et al.*, 1995) and evaporative demand (Dunlap and Helms, 1983; Aussenac, 2000), it is possible that the decrease in  $\delta^{18}\text{O}$  that would be expected if differences in  $E$  were driven by changes in  $g_s$  could have been masked by an increase in  $\delta^{18}\text{O}$  driven by greater evaporative demand in VRH treatments relative to overstorey controls. The nonsignificant increases in  $\delta^{18}\text{O}$  from controls to VRH treatments for *P. banksiana* and *P. resinosa* could suggest greater  $g_s$  in the retention treatments than in the overstorey controls coupled with either a corresponding increase in  $A_{\text{max}}$  or evaporative demand (Barbour *et al.*, 2002). Gas exchange measurements also indicated higher  $g_s$  in several neighbourhood environments created by VRH compared with the closed canopy environment characteristic of reference stands. However, any increase in  $g_s$  associated with VRH must have been paralleled by a considerable increase in carbon assimilation to draw down  $c_i$  and produce the observed differences in  $\delta^{13}\text{C}$ . Seedlings were also much larger in VRH treatments, which is consistent with greater carbon assimilation.

Our measured rates of  $A_{\text{max}}$  and  $g_s$  were similar to values reported in other studies at similar latitudes for *P. resinosa* and *P. banksiana* seedlings (Parker and Mohammed, 2000;

Powers *et al.*, 2008b), but our  $A_{\text{max}}$  values are 25–50 per cent lower than values reported for *P. strobus* seedlings (Boucher *et al.*, 2001, 2007) despite a similar range of  $g_s$ . While a number of methodological issues can make it difficult to collect accurate gas exchange measurements on the relatively small amounts of leaf area we were enclosing in the LI-6400 cuvette, we can think of none that would affect *P. strobus* without also impacting our other study species.

## Conclusions

Our findings indicate average seedling development in northern pine forests is similar following retention harvests that produce stand structures ranging from evenly dispersed residual trees to stands with residual trees aggregated between large (0.3 ha) silvicultural gaps. Although VRH appeared to substantially increase photosynthetic capacity, doubled seedling size and may have increased  $g_s$  compared with unharvested stands, we found no consistent evidence of differences in average stand-scale seedling physiological performance or growth associated with different spatial patterns of overstorey retention. Species ranging from very intolerant (*P. banksiana*) to moderately tolerant (*P. strobus*) of shade showed similar responses to VRH treatments using dispersed retention, aggregated retention between 0.1 ha gaps and aggregated retention between 0.3 ha gaps.

These results have important implications for natural resource managers. The absence of large differences among VRH treatments suggests seedling growth and productivity can be similar across stands with very different, and potentially complex, overstorey structures when averaged across whole stands. While other studies suggest the potential for neighbourhood-scale differences in seedling development within VRH treatments, this potential did not appear to translate into differences in treatment means in our study system when seedling performance was averaged across all of the neighbourhood environments in a given treatment.

We do caution that these results are based on data from only the fourth (isotopes, growth) and fifth (gas exchange) growing season after harvesting, and differences in seedling ecophysiology may manifest as stand development continues. Nevertheless, our results suggest forest managers interested in increasing tree species diversity and structural complexity in managed forests have considerable flexibility to adopt VRH systems that use different patterns of overstorey retention but support similar stand-scale rates of early seedling development.

## Funding

United State Department of Agriculture Forest Service, Northern Research Station and Michigan Technological University.

## Acknowledgements

We would like to thank Linda Nagel, Tom Drummer, Shawn Fraver, Rebecca Montgomery, and Peter Reich for their valuable input during the development of this project. Two anonymous



reviewers provided useful comments on an earlier version of this paper. Julia Robinson assisted with sample preparation for stable isotope and elemental analysis. Jennifer Eikenberry and Benjamin Harlow conducted our stable isotope analyses.

### Conflict of interest statement

None declared.

### References

- Acker, S.A., Zenner, E.K. and Emmingham, W.H. 1998 Structure and yield of two-aged stands on the Willamette National Forest, Oregon: implications for green tree retention. *Can. J. For. Res.* **28**, 749–758.
- Aussenac, G. 2000 Interactions between forest stands and microclimate: ecophysiological aspects and consequences for silviculture. *Ann. For. Sci.* **57**, 287–301.
- Aussenac, G. and Granier, A. 1988 Effects of thinning on water stress and growth in Douglas-fir. *Can. J. For. Res.* **18**, 100–105.
- Barbour, M.M. 2007 Stable oxygen isotope composition of plant tissue: a review. *Funct. Plant Biol.* **34**, 83–94.
- Barbour, M.M., Walcroft, A.S. and Farquhar, G.D. 2002 Seasonal variation in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of cellulose from growth rings of *Pinus radiata*. *Plant Cell Environ.* **25**, 1483–1499.
- Bond, B.J., Farnsworth, B.T., Coulombe, R.A. and Winner, W.E. 1999 Foliage physiology and biochemistry in response to light gradients in conifers with varying shade tolerance. *Oecologia* **120**, 183–192.
- Boucher, J.F., Bernier, P.Y. and Munson, A.D. 2001 Radiation and soil temperature interactions on the growth and physiology of eastern white pine (*Pinus strobus* L.) seedlings. *Plant Soil* **236**, 165–174.
- Boucher, J.F., Bernier, P.Y., Margolis, H.A. and Munson, A.D. 2007 Growth and physiological response of eastern white pine seedlings to partial cutting and site preparation. *For. Ecol. Manage.* **240**, 151–164.
- Breda, N., Granier, A. and Aussenac, G. 1995 Effects of thinning on soil water balance and tree water relations, transpiration, and growth in oak forest (*Quercus petraea* (Matt) Liebl.). *Tree Physiol.* **15**, 295–306.
- Burgess, D. and Wetzel, S. 2000 Nutrient availability and regeneration response after partial cutting and site preparation in eastern white pine. *For. Ecol. Manage.* **138**, 249–261.
- Coates, K.D. and Burton, P.J. 1997 A gap-based approach for development of silvicultural systems to address ecosystem management objectives. *For. Ecol. Manage.* **99**, 337–354.
- Comstock, J. and Ehleringer, J.R. 1984 Photosynthetic responses to slowly decreasing leaf water potentials in *Encelia frutescens*. *Oecologia* **61**, 241–248.
- Dang, Q.L., Margolis, H.A., Coyea, M.R., Sy, M. and Collatz, G.J. 1997 Regulation of branch-level gas exchange of boreal trees: roles of shoot water potential and vapor pressure difference. *Tree Physiol.* **17**, 521–535.
- DeNiro, M.J. and Epstein, S. 1979 Relationship between the oxygen isotope ratios of terrestrial plant cellulose, carbon dioxide, and water. *Science* **204**, 51–53.
- Dongmann, G., Nurnberg, H.W., Forstel, H. and Wagener, K. 1974 On the enrichment of  $\text{H}_2^{18}\text{O}$  in the leaves of transpiring plants. *Radiat. Environ. Biophys.* **11**, 41–52.
- Dumais, D. and Prevost, M. 2008 Ecophysiology and growth of advance red spruce and balsam fir regeneration after partial cutting in yellow birch-conifer stands. *Tree Physiol.* **29**, 1221–1229.
- Dunlap, J.M. and Helms, J.A. 1983 First-year growth of planted Douglas-fir and white fir seedlings under different shelterwood regimes in California. *For. Ecol. Manage.* **5**, 255–268.
- Ellsworth, D.S. and Reich, P.B. 1993 Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia* **96**, 169–178.
- Evans, J.R. 1983 Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). *Plant Physiol.* **72**, 297–302.
- Farquhar, G.D. and Lloyd, J. 1993 Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In *Stable Isotopes and Plant Carbon/Water Relations*. J.R. Ehleringer, A.E. Hall and G.D. Farquhar (eds). Academic Press, New York, NY, pp. 49–79.
- Farquhar, G.D., O'Leary, M.H. and Berry, J.A. 1982 On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J. Plant Physiol.* **9**, 121–137.
- Farquhar, G.D., Ehleringer, J.R. and Hubick, K.T. 1989 Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40**, 503–537.
- Farquhar, G.D., Cernusak, L.A. and Barnes, B. 2007 Heavy water fractionation during transpiration. *Plant Physiol.* **143**, 11–18.
- Franklin, J.F., Lindenmayer, D.B., MacMahon, J.A., McKee, A., Magnusson, J. and Perry, D.A. *et al.* 2000 Threads of continuity: ecosystem disturbances, biological legacies and ecosystem recovery. *Conserv. Biol. Pract.* **1**, 8–16.
- Franklin, J.F., Spies, T.A., Van Pelt, R., Carey, A.B., Thornburgh, D.A. and Berg, D.R. *et al.* 2002 Disturbances and structural development of natural forest ecosystems with silvicultural implications, using Douglas-fir forests as an example. *For. Ecol. Manage.* **155**, 399–423.
- Halpern, C.B., Evans, S.A., Nelson, C.R., McKenzie, D., Ligouri, D.A. and Hibbs, D.E. *et al.* 1999 Response of forest vegetation to varying levels and patterns of green-tree retention: an overview of a long-term experiment. *Northwest Sci.* **73**, 27–44.
- Johnson, J.D. 1984 A rapid technique for estimate total surface area of pine needles. *For. Sci.* **30**, 913–921.
- Leavitt, S.W. and Long, A. 1984 Sampling strategy for stable carbon isotope analysis of tree rings in pine. *Nature* **311**, 145–147.
- Leavitt, S.W. and Long, A. 1986 Stable-carbon isotope variability in tree foliage and wood. *Ecology* **67** (4): 1002–1010.
- Maguire, D.A., Mainwaring, D.B. and Halpern, C.B. 2006 Stand dynamics after variable-retention harvesting in mature Douglas-fir forests of western North America. *Allg. Forst. Jagdztg.* **177**, . Jg., 6/7, 120–121.
- Maier, C.A. and Teskey, R.O. 1992 Internal and external control of net photosynthesis and stomatal conductance of mature eastern white pine (*Pinus strobus*). *Can. J. For. Res.* **22**, 1387–1394.
- McGuire, J.P., Mitchell, R.J., Moser, E.B., Pecot, S.D., Gjerstad, D.H. and Hedman, C.W. 2001 Gaps in a gappy forest: plant resources, longleaf pine regeneration and understory response to tree removal in longleaf pine savannas. *Can. J. For. Res.* **31**, 765–778.
- Mitchell, A.K., Koppenaal, R., Goodmanson, G., Benton, R. and Bown, T. 2007 Regenerating montane conifers with variable

- retention systems in a coastal British Columbia forest: 10-year results. *For. Ecol. Manage.* **246**, 240–250.
- Nagel, L.M. and O'Hara, K.L. 2001 The influence of stand structure on ecophysiological leaf characteristics of *Pinus ponderosa* in western Montana. *Can. J. For. Res.* **31**, 2173–2182.
- Oguchi, R., Hikosaka, K., Hiura, T. and Hirose, T. 2006 Leaf anatomy and light acclimation in woody seedlings after gap formation in a cool-temperate deciduous forest. *Oecologia*. **149**, 571–582.
- Palik, B.J., Mitchell, R.J., Houseal, G. and Pederson, N. 1997 Effects of canopy structure on resource availability and seedling responses in a longleaf pine ecosystem. *Can. J. For. Res.* **27**, 1458–1464.
- Palik, B.J., Mitchell, R.J. and Hiers, J.K. 2002 Modeling silviculture after natural disturbance to sustain biodiversity in the longleaf pine (*Pinus palustris*) ecosystem: balancing complexity and implementation. *For. Ecol. Manage.* **155**, 347–356.
- Palik, B.J. and Zasada, J. 2003 *An Ecological Context for Regenerating Multi-cohort, Mixed Species Red Pine Forests*. USDA Forest Service. Research Paper NC-382, St Paul, MN.
- Palik, B.J., Mitchell, R.J., Pecot, S., Battaglia, M. and Pu, M. 2003 Spatial distribution of overstory retention influences resources and growth of longleaf pine seedlings. *Ecol. Appl.* **13**, 674–686.
- Palik, B.J., Kern, C., Mitchell, R.J. and Pecot, S. 2005 *Using Spatially Variable Overstory Retention to Restore Structural and Compositional Complexity in Pine Ecosystems*. USDA Forest Service. General Technical Report PNW-GTR-635, Portland, OR.
- Parker, W.C. and Mohammed, G.H. 2000 Photosynthetic acclimation of shade-grown red pine (*Pinus resinosa* Ait.) seedlings to a high light environment. *New For.* **19**, 1–11.
- Pothier, D. and Prevost, M. 2002 Photosynthetic light response and growth analysis of competitive regeneration after partial cutting in a boreal mixed stand. *Trees*. **16**, 365–373.
- Powers, M.D., Pregitzer, K.S. and Palik, B.J. 2008a  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  trends across overstory environments in whole foliage and cellulose of three *Pinus* species. *J. Am. Soc. Mass Spectrom.* **19**, 1330–1335.
- Powers, M.D., Pregitzer, K.S. and Palik, B.J. 2008b Physiological performance of three pine species provides evidence for gap partitioning. *For. Ecol. Manage.* **256**, 2127–2135.
- Powers, M.D., Pregitzer, K.S., Palik, B.J. and Webster, C.R. 2009 Water relations of pine seedlings in contrasting overstory environments. *For. Ecol. Manage.* **258**, 1442–1448.
- Prescott, C.E., Hope, G.D. and Blevins, L.L. 2003 Effect of gap size on litter decomposition and soil nitrate concentrations in a high-elevation spruce-fir forest. *Can. J. For. Res.* **33**, 2210–2220.
- Reich, P.B., Kloeppel, B., Ellsworth, D.S. and Walters, M.B. 1995 Different photosynthesis-nitrogen relations in deciduous hardwood and evergreen coniferous tree species. *Oecologia*. **104**, 24–30.
- Richardson, T.N. 1997 *Soil Survey of Cass County, Minnesota*. USDA NRCS and Forest Service., St. Paul, MN.
- Ritter, E., Dalsgaard, L. and Einhorn, K.S. 2005 Light, temperature and soil moisture regimes following gap formation in a semi-natural beech-dominated forest in Denmark. *For. Ecol. Manage.* **206**, 15–33.
- Seibt, U., Rajabi, A., Griffiths, H. and Berry, J.A. 2008 Carbon isotopes and water use efficiency: sense and sensitivity. *Oecologia*. **155**, 441–454.
- Skov, K.R., Kolb, T.E. and Wallin, K.F. 2004 Tree size and drought affect ponderosa pine physiological response to thinning and burning treatments. *For. Sci.* **50**, 81–91.
- Sullivan, T.P., Sullivan, D.S. and Lindgren, P.M.F. 2008 Influence of variable retention harvests on forest ecosystems: Plant and mammal responses up to 8 years post-harvest. *For. Ecol. Manage.* **254**, 230–254.
- Svenson, S.E. and Davies, F.T. 1992 Comparison of methods for estimating surface area of water-stressed and fully hydrated pine needle segments for gas exchange analysis. *Tree Physiol.* **10**, 417–421.
- Wetzel, S. and Burgess, D. 2001 Understorey environment and vegetation response after partial cutting and site preparation in *Pinus strobus* L. stands. *For. Ecol. Manage.* **151**, 43–59.
- White, H. 1980 A heteroskedasticity-consistent covariance estimator and a direct test for heteroskedasticity. *Econometrica*. **48**, 817–838.
- Yakir, D. 1992 Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. *Plant Cell Environ.* **15**, 1005–1020.

Received 23 April 2010